

CLAIMS

We claim:

1. A buffered solution for multiplexed binding assays using GPCR arrays, the
5 solution having a composition comprising: a) a buffer reagent with a pH in the range of about 6.5 to about 7.9; b) an inorganic salt of either a monovalent or divalent species, at a concentration from about 1 mM to about 500 mM; and optionally a combination of: c) a blocker reagent at a concentration of about 0.01 wt.% to about 2 wt.% of the
10 composition, or d) protease-inhibitor at a concentration of about 0.001 mM to about 100 mM, or both c) and d).
2. The buffered solution according to claim 1, wherein said pH is in a range of about 6.8-7.8.
- 15 3. The buffered solution according to claim 1, wherein said pH is about 7.4-7.5.
4. The buffered solution according to claim 1, wherein when said inorganic salt is a monovalent species, said concentration of said salt is about 10-500 mM.
- 20 5. The buffered solution according to claim 1, wherein when said inorganic salt is a divalent species, said concentration of said salt is about 1-50 mM.
6. The buffered solution according to claim 1, wherein said composition further comprising: a labeled ligand and a target compound.
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7. The buffered solution according to claim 1, wherein said pH buffer is made from a solution having commonly used pH control reagents selected from Tris-HCl, HEPES-KOH, TES-NH₄OH, MOPS, acetate, citrate, citrate-phosphate, sodium-phosphate, maleate, or succinate buffers.
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8. The buffered solution according to claim 1, wherein said inorganic salt may be selected from NaCl, KCl, CaCl₂, MgCl₂, MgSO₄, or MnCl₂.

9. The buffered solution according to claim 1, wherein said blocker reagent is a hydrophilic polymer, a biopolymer, or a water-soluble protein

5 10. The buffered solution according to claim 9, wherein said blockers characterized as a reagent that reduces background signal and does not interfere with the binding of a target molecule with the probe receptors within a biological membrane microspot.

10 11. The buffered solution according to claim 9, wherein said hydrophilic polymer is dextran, polyvinyl alcohol, poly (ethylene glycol), poly(anetholsulfate), poly(vinyl sulfate), CM-Dextran, dextran sulfate, beta-cyclodextrin, poly(acrylic acid), poly(sodium 4-styrene sulfonate).

15 12. The buffered solution according to claim 9, wherein said biopolymer is poly-glutamate acid, or DNA.

13. The buffered solution according to claim 9, wherein said water-soluble protein is bovine serum albumin (BSA), casein, dry milk, or wheat germ agglutinin.

20 14. The buffered solution according to claim 1, wherein said solution is protease-free.

25 15. The buffered solution according to claim 1, wherein said protease inhibitor may include EDTA, EGTA, phenyl methyl sulfonyl fluoride (PMSF), bacitracin, 4-(2-aminoethyl)benzenesulfonyl fluoride (AEBSF), 1,10-phenanthroline, E-64, antipain, aprotinin, benzamidine HCl, bestatin, chymostatin, ϵ -aminocaproic acid, N-ethylmaleimid, leupeptin, pepstatin A, phosphoramidon, trypsin inhibitor, and any combination of these.

30 16. A buffered solution for functional assays according to a GTP-analogue-binding profile approach, the solution having a composition comprising: a) a buffer reagent with a pH in the range of about 6.5 to about 7.9; b) a divalent inorganic salt, optionally together with a monovalent inorganic salt, at a concentration from about 1 mM to about

500 mM; c) guanosine 5'-diphosphate (GDP) salt at a concentration of about 0.5 mM to about 50 mM (1-10 mM); and optionally a combination of: d) a blocker reagent at a concentration of about 0.01 wt.% to about 2 wt.% of the composition, e) protease-inhibitor at a concentration of about 0.001 mM to about 100 mM, or f) an anti-oxidant reagent at a concentration of 0.01 mM to about 100 mM.

17. The solution according to claim 16, wherein said GTP-analogue includes fluorescein-GTP γ S, Bodipy-fluorescein-GTP γ S, Bodipy-TMR-GTP γ S, Cy3-GTP γ S, Cy5-GTP γ S, Eu-GTP, 35 S-GTP γ S.

18. The solution according to claim 16, wherein said GDP salt is selected from a group consisting of: lithium-, sodium-, and Tris-GDP salts.

19. The solution according to claim 16, wherein said anti-oxidant reagent includes sodium ascorbate, ascorbic acid, carotenoid lycopene, α -tocopherol, β -carotene, sodium azide.

20. The solution according to claim 16, wherein said anti-oxidant reagent has a concentration in a range of about 0.001 wt.% to about 0.5 wt. %

21. The solution according to claim 16, wherein said pH is in a range of about 6.8-7.8.

22. The solution according to claim 18, wherein said pH is about 7.4-7.5.

23. The solution according to claim 16, wherein said pH buffer is made from a solution having commonly used pH control reagents selected from Tris-HCl, HEPES-KOH, TES-NH₄OH, MOPS, acetate, citrate, citrate-phosphate, sodium-phosphate, maleate, or succinate buffers.

24. The solution according to claim 16, wherein said inorganic salt may be selected from NaCl, KCl, CaCl₂, MgCl₂, MgSO₄, or MnCl₂.

25. The solution according to claim 16, wherein said blocker reagent is a hydrophilic polymer, a biopolymer, or a water-soluble protein.

5 26. The solution according to claim 22, wherein said blockers characterized as a reagent that reduces background signal and does not interfere with the binding of a target molecule with the probe receptors within a biological membrane microspot.

10 27. The solution according to claim 22, wherein said hydrophilic polymer is dextran, polyvinyl alcohol, poly (ethylene glycol), poly(anetholsulfate), poly(vinyl sulfate), CM-Dextran, dextran sulfate, beta-cyclodextrin, poly(acrylic acid), poly(sodium 4-styrene sulfonate).

15 28. The solution according to claim 22, wherein said biopolymer is poly-glutamate acid, or DNA.

29. The solution according to claim 22, wherein said water-soluble protein is bovine serum albumin (BSA), casein, dry milk, or wheat germ agglutinin.

20 30. The solution according to claim 16, wherein said solution is protease-free.

25 31. The solution according to claim 16, wherein said protease inhibitor may include EDTA, EGTA, phenyl methyl sulfonyl fluoride (PMSF), bacitracin, 4-(2-aminoethyl)benzenesulfonyl fluoride (AEBSF), 1,10-phenanthroline, E-64, antipain, aprotinin, benzamidine HCl, bestatin, chymostatin, ϵ -aminocaproic acid, N-ethylmaleimid, leupeptin, pepstatin A, phosphoramidon, trypsin inhibitor, and any combination of these.

30 32. A method of reducing background signal due to non-specific binding of a labeled-ligand or GTP-analogue to a substrate surface, the method comprising: a) providing a buffered solution containing a blocker reagent; b) applying said solution to an array of GPCRs; c) applying a second solution containing a labeled ligand or GTP-

analogue, in either the absence or presence of a target compound; and d) monitoring or determining the binding of said labeled ligand to a receptor, or said GTP-analogue to a G-protein coupled with said receptor in said array.

5 33. The method according to claim 30, wherein said method further comprises a washing and dry step before data acquisition.

10 34. A method of reducing background signal due to non-specific binding of a labeled-ligand or GTP-analogue to a substrate surface, the method comprising: a) providing a solution containing a blocker reagent and a labeled ligand or GTP-analogue, in either the absence or presence of a target compound; b) applying said solution to a microarray of GPCRs; and c) monitoring or determining the binding of said labeled ligand to a receptor, or said GTP-analogue to a G-protein coupled with said receptor in said microarray.

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